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Shoot-to-Root Signal Transmission Regulates Root Fe(III) Reductase Activity in the *dgl* Mutant of Pea[1]

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To understand the root, shoot, and Fe-nutritional factors that regulate root Fe-acquisition processes in dicotyledonous plants, Fe(III) reduction and net proton efflux were quantified in root systems of an Fe-hyperaccumulating mutant (*dgl*) and a parental (cv Dippes Gelbe Viktoria [DGV]) genotype of pea (*Pisum sativum*). Plants were grown with (+Fe treated) or without (−Fe treated) added Fe(III)-*N,N'*-ethylenebis[2-(2-hydroxyphenyl)-glycine] (2 μM); root Fe(III) reduction was measured in solutions containing growth nutrients, 0.1 mM Fe(III)-ethylenediaminetetraacetic acid, and 0.1 mM Na_2-bathophenanthrolinedisulfonic acid. Daily measurements of Fe(III) reduction (d 10–20) revealed initially low rates in +Fe-treated and −Fe-treated *dgl*, followed by a nearly 5-fold stimulation in rates by d 15 for both growth types. In DGV, root Fe(III) reductase activity increased only minimally by d 20 in +Fe-treated plants and about 3-fold in −Fe-treated plants, beginning on d 15. Net proton efflux was enhanced in roots of −Fe-treated DGV and both *dgl* growth types, relative to +Fe-treated DGV. In *dgl*, the enhanced proton efflux occurred prior to the increase in root Fe(III) reductase activity. Reductase studies using plants with reciprocal shoot:root grafts demonstrated that shoot expression of the *dgl* gene leads to the generation of a transmissible signal that enhances Fe(III) reductase activity in roots. The *dgl* gene product may alter or interfere with a normal component of a signal transduction mechanism regulating Fe homeostasis in plants.

The process of root Fe acquisition in dicotyledonous and nongraminaceous monocotyledonous plants involves the reduction of ferric to ferrous Fe, prior to the root-membrane influx of Fe^{2+} (Chaney et al., 1972). Our current understanding of root Fe(III) reduction, which has been investigated in a number of species (Moog and Brüggemann, 1994), is that a plasmalemma-localized reductase system is primarily responsible (Buckhout et al., 1989; Grusak et al., 1989; Brüggemann et al., 1990; Holden et al., 1991). Various organic compounds released from roots also can reduce Fe(III), but in most cases these appear to account for only a minor amount of Fe^{2+} generation (Römheld and Marschner, 1983). In pea (*Pisum sativum* L.), the root Fe(III) reductase appears to be the rate-limiting step in the overall Fe-acquisition process (Grusak et al., 1990b) and thus is critical to the general Fe nutrition of the plant. Much research has recently focused, therefore, on the functional characterization and regulation of this important enzyme (Chaney, 1989; Cornett and Johnson, 1991; Holden et al., 1992, 1994; Schmidt, 1994a, 1994b).

Although we have known for some time that Fe-deficiency stress can stimulate root Fe(III) reductase activity (Römheld and Marschner, 1986; Kochian, 1991), it is still unclear how this system is regulated at the cytoplasmic or whole-plant levels. Studies with potato (*Solanum tuberosum* L.) have indicated that the control of the reductase and other Fe-related processes may be located within the roots themselves (Bienfait et al., 1987); it has been postulated that the reductase might be transcriptionally regulated by the product of the FER gene, which is thought to be an Fe-responsive regulatory protein (Bienfait, 1988). The regulatory activity of the FER protein would presumably be dependent on the extracellular Fe supply (and the influx and utilization of Fe^{2+}); however, limited rhizospheric Fe may not be the primary stimulus or the only stimulus in this control process. A recent study of pea, in which the plants were continuously maintained on an adequate Fe source, revealed dramatic modulations in root Fe(III) reductase activity throughout the plant's life cycle (Grusak, 1995); reductase activity increased and then decreased during the seed fill period. These changes were shown to be internally regulated and involved the transmission of a phloem-mobile signal to the roots from the reproductive region of the shoot; this signal presumably conveyed information relevant to the shoot's Fe status. Other investigators have also provided evidence for the involvement of shoot-to-root communication in the regulation of root Fe(III) reductase activity, suggesting that the signal might be a hormone (Landsberg, 1984) or recirculated Fe (Maas et al., 1988; Bienfait, 1989).

Previously, studies with the *brz* mutant of pea (Grusak et al., 1990a) and the *chloronerva* mutant of tomato (*Lycopersicon esculentum* L; Stephan and Grün, 1989) also showed that root Fe(III) reductase activity can be elevated, relative to

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Abbreviations: BPDS, bathophenanthrolinedisulfonic acid; DGV, Dippes Gelbe Viktoria; EDDHA, *N,N'*-ethylenebis[2-(2-hydroxyphenyl)-glycine].

control genotypes, even when the mutant plants are maintained on an Fe source. Because of the value these and other mutants have shown in enhancing our understanding of higher plant Fe nutrition (Yi et al., 1994), and because of the need to identify the internal signals that regulate the reductase, we have undertaken a study of various root and shoot processes relevant to the Fe physiology of the *dgl* mutant of pea. This mutant, first noted by Gottschalk (1987) to have brown, degenerative leaves, has been shown to have the capacity to overaccumulate Fe in its vegetative tissues (Kneen et al., 1990). In this paper, data will be presented concerning +Fe- and −Fe-treated plants of the *dgl* mutant and its parent genotype DGV; we describe their root capacity for Fe reduction and for acidification of the rhizosphere. Results of reciprocal grafting experiments also will be presented to discuss whether shoot tissues are involved in the internal regulation of root Fe(III) reductase activity in both the *dgl* mutant and the *brz* pea mutant.

MATERIALS AND METHODS

Plant Material and Culture

Four genotypes of pea (*Pisum sativum* L.) were used in the present study: a parent line (cv DGV) and a derived mutant line (cv DVG [*dgl dgl*]), which was generated via x-ray-induced mutagenesis of cv DGV (Gottschalk, 1987), as well as a second parent line (cv Sparkle) and a mutant line (cv Sparkle [*brz brz*]), which was generated via ethyl methanesulfonic acid mutagenesis of cv Sparkle (Kneen et al., 1990). The *brz* mutant previously was referred to as the E107 pea mutant (Grusak et al., 1990a, 1993); note that we will refer to it as *brz* throughout this paper.

For plant growth, seeds were allowed to imbibe on d 0, planted on d 3, and grown hydroponically in polyethylene vessels containing 3.5 L of aerated nutrient solution as previously described (Grusak et al., 1990a). Nutrient solutions contained the following macronutrients in mM: KNO_3, 1.2; $Ca(NO_3)_2$, 0.8; $NH_4H_2PO_4$, 0.3; $MgSO_4$, 0.2; and the following micronutrients in μM: $CaCl_2$, 25; H_3BO_3, 25; $MnSO_4$, 2; $ZnSO_4$, 2; $CuSO_4$, 0.5; H_2MoO_4, 0.5; $NiSO_4$, 0.1. Nutrient solutions were replaced on d 8, 10, 12, 14, 16, 18, and 19 and were buffered with Mes (adjusted with KOH), which maintained the pH between 5.4 and 5.8; buffer strength was 1 mM until d 12 and 2 mM thereafter. Plants were grown either with 2 μM Fe(III)-EDDHA (+Fe-treated plants; Fe initially presented on d 3) or with no added Fe (−Fe-treated plants). Fe(III)-EDDHA was prepared according to the directions of Chaney and Bell (1987), using KOH for pH adjustment. Plants were grown in a controlled environmental chamber with a 16-h, 20°C/8-h, 15°C day/night regime under a mixture of incandescent and fluorescent lamps; intensity of PAR was 350 μmol photons m^{-2} s^{-1} at the level of the lowest leaf.

Experimental Techniques

Root-associated Fe(III) reduction was determined for whole, excised root systems using the spectrophotometric measurement of Fe(II) chelated to BPDS as previously described (Grusak et al., 1990b); the assay solution consisted of the macronutrients and micronutrients listed above, along with 1 mM Mes buffer (pH 5.5), 0.1 mM Fe(III)-EDTA, and 0.1 mM Na_2-BPDS. Assays were conducted in a darkened laboratory at 22°C for 20 min; preliminary studies confirmed that Fe(III) reduction in the excised roots was linear for up to 60 min (data not shown). A_{535} values of the assay solutions were determined spectrophotometrically with a Gilford Response II, UV-VIS spectrophotometer (Ciba-Corning Diagnostics, Medfield, MA); an assay solution sample without roots, exposed to the same ambient conditions as solutions with roots, was used as a blank. Fe(II)-$BPDS_3$ was quantified using a molar extinction coefficient of 22.14 mM^{-1} cm^{-1}. Following each assay, roots were gently blotted dry and fresh weights were determined; all reductase values were calculated on a fresh weight basis. The amount of Fe(III) reduction attributable to root reductant release was determined as previously described (Grusak et al., 1990a). In selected roots, Fe(III) reductase activity was visualized using the agarose-BPDS technique (Grusak et al., 1993).

Root Fe(III) reductase activity also was measured in root systems of grafted plants, which were maintained on Fe(III)-EDDHA. Hydroponically grown seedlings were selected soon after emergence (d 7–8); tissues were grafted at the stem directly above the cotyledonary node. For grafting, stems were cut on a 45° angle (relative to the edge of the stem) with a razor blade; grafts were held together with a short length of plastic tubing. The grafted plants were returned to nutrient solution (with 2 μM Fe[III]-EDDHA) as described above and were immediately placed in a plexiglass box whose internal atmosphere was maintained at approximately 100% RH with multiple airstones bubbling in beakers of distilled H_2O. Plants were kept in dim light (15 μmol photons m^{-2} s^{-1}, PAR) for 3 to 5 d until the graft was established; light intensity was then gradually increased and RH was gradually decreased throughout a 3-d period before the plants were returned to normal growing conditions. Throughout the graft establishment and recovery period, all side shoots originating near the cotyledons (i.e. below the graft union) were removed at first appearance with scissors; cotyledons were covered with black polyethylene beads during and after this period. In one series of experiments, however, a single side shoot originating below the graft union was allowed to remain so that plants with two shoots and one root system were generated. Reductase assays were performed using excised, whole-root systems after the plants had attained a size comparable to nongrafted plants at d 15 to 18. Self-grafts, composed of shoots and roots of the same genotype, always utilized tissues from different seedlings.

Root capacity for rhizosphere acidification was measured during two time intervals: d 10 to 11 and d 14 to 15. In each case, groups of four plants were transferred to fresh nutrient solution (3.5 L), with or without Fe, and contained 1 or 2 mM Mes; all studies began between the 4th and 5th h of the light period. Twenty-four hours later, the plants were removed, the root systems were excised, and root fresh weights were determined. Samples of nutrient solution were taken from each container after the volume was

brought back to 3.5 L with distilled H_2O (when necessary). Aliquots of both collected samples and initial solution samples were titrated to pH 8.0 using a KOH standard, and molar differences were determined. This termination point (pH 8.0) was chosen to improve our titration resolution because it is outside the useful buffer range for Mes buffer. Net rates of proton efflux for the 24-h period were calculated on a gram fresh weight basis using the combined root fresh weight of the four plants at the time of harvest.

All reported reductase values were based on a minimum of six root systems, and proton efflux rates were based on at least three containers of four plants. Statistical significance of differences between mean values was determined using Student's *t* test.

RESULTS

Daily measurements of root Fe(III) reductase activity revealed consistently higher rates in the *dgl* mutant, relative to its parent genotype DGV (Fig. 1). Both +Fe-treated and −Fe-treated *dgl* exhibited low reductase rates on d 10 and 11, followed by increasing rates throughout the remainder of the 20-d study period; the timing of the rate increase was delayed in −Fe-treated *dgl*, relative to +Fe-treated *dgl*. Reductase values of +Fe-treated *dgl* were always higher than or equal to those of −Fe-treated *dgl*. In DGV, +Fe-treated plants maintained low reductase rates throughout the first 20 d of growth, whereas −Fe-treated plants exhibited significantly higher rates starting on d 15 and continuing through d 20.

Fe(III) reduction due to the release of soluble reductants was measured in root systems of d 15 plants of all DGV and *dgl* growth types. Released reductants accounted for only 0 to 4% of total root Fe(III) reductase activity, indicating that the membrane-associated reductase system was primarily responsible for all measured activity. Root localization of Fe(III) reductase activity was also studied in primary lateral roots of d 15 plants. Extensive reducing regions were found from the base of primary lateral roots to within 2 to 4 cm of the apex in +Fe-treated *dgl*, −Fe-treated *dgl*, and

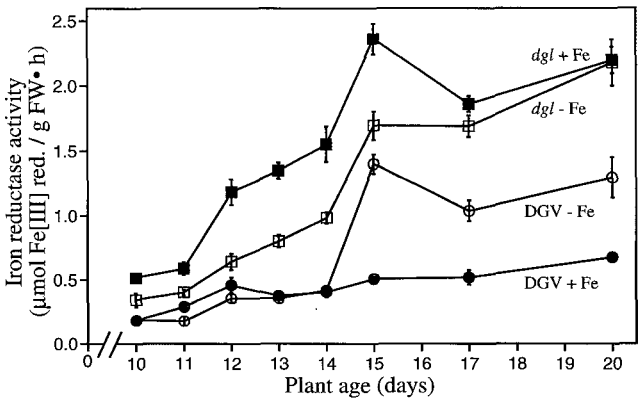

Figure 1. Root Fe(III) reductase activity measured in excised root systems of +Fe-treated and −Fe-treated pea plants. +Fe-treated plants were maintained on 2 μM Fe(III)-EDDHA during growth; assays were conducted using 0.1 mM Fe(III)-EDTA. Values are means based on at least six plants; bars represent SE. FW, Fresh weight.

Table I. *Rates of net proton efflux from root systems of pea genotypes maintained with or without Fe*

Proton efflux was measured in buffered nutrient solution during a 24-h period. Values are means ± SE and are based on five containers (*dgl*, d 10–11 values) or three containers (all other treatment values) of four plants.

Growth Type	d 10–11	d 14–15
	μmol H^+ efflux g^{-1} fresh wt h^{-1}	
DGV + Fe	2.90 ± 0.05	2.00 ± 0.23
DGV − Fe	2.19 ± 0.20	8.71 ± 1.24
dgl + Fe	7.29 ± 0.22	9.82 ± 1.23
dgl − Fe	5.01 ± 0.29	12.19 ± 1.02

−Fe-treated DGV; primary lateral roots of +Fe-treated DGV showed less extensive, sometimes patchy staining regions, again with no staining at the apical 2 to 4 cm (photographic data not shown).

Because roots of Fe-deficient plants previously have been shown to have a stimulated efflux of protons (Römheld and Marschner, 1986), this phenomenon was investigated in +Fe-treated and −Fe-treated plants of *dgl* and DGV (Table I). Net proton efflux was significantly higher in the *dgl* growth types, relative to the DGV growth types, during the d 10 to 11 measurement period; the elevated proton efflux preceded the enhanced Fe(III) reductase activities observed in this mutant (cf. Fig. 1). By the d 14 to 15 time interval, however, only +Fe-treated DGV showed a low rate of proton efflux, which was significantly lower than that of all other growth types (Table I). Furthermore, net proton efflux rates were significantly higher for −Fe-treated DGV and −Fe-treated *dgl* during the d 14 to 15 study period, relative to the d 10 to 11 study period.

To determine whether the genetic mutation conditioning the enhanced Fe(III) reductase activity in roots of the *dgl* mutant affects root tissues directly or indirectly via an alteration in the shoots, we measured Fe(III) reduction rates in root systems of grafted plants maintained on 2 μM Fe(III)-EDDHA (Table II). Self-grafts, composed of shoot and root tissues of common genotypic origin, all showed anticipated root Fe(III) reductase rates: DGV and Sparkle self-grafts exhibited low activities, whereas *dgl* and *brz* self-grafts exhibited elevated activities. In reciprocal graft combinations, root Fe(III) reductase rates were elevated whenever the shoot tissues were provided by the mutant genotype (either *dgl* or *brz*); for grafted plants comprising mutant roots and parent genotype shoots (e.g. Sparkle/*brz*), root Fe(III) reductase rates were not elevated. Unfortunately, attempts to graft DGV shoots onto *dgl* roots were unsuccessful under current growth conditions, and no data are available for this graft combination. Additionally, when both a *dgl* and DGV shoot were simultaneously connected to a DGV root system, root Fe(III) reductase activity was elevated in the wild-type root (Table II).

Having demonstrated a role for *dgl* shoot tissues in the enhancement of root Fe(III) reductase activity, we investigated whether the gradual daily increase in this activity (Fig. 1) might be related to a particular shoot tissue. Specifically, we wondered whether the transition in shoot-to-root nutrition based first on stored reserves of the cotyle-

Table II. *Root Fe(III) reductase activity in grafted plants*

Activity was measured using 0.1 mM Fe(III)-EDTA in plants that had attained a size comparable to 15- to 18-d-old nongrafted plants. Plants were maintained continuously on 2 μM Fe(III)-EDDHA during growth. Values are means ± SE, $n \geq 6$.

Shoot/Root	μmol Fe(III) reduced g^{-1} fresh wt h^{-1}
DGV/DGV	0.53 ± 0.08
dgl/dgl	1.48 ± 0.16
Sparkle/Sparkle	0.30 ± 0.08
brz/brz	0.99 ± 0.06
dgl/DGV	1.32 ± 0.16
brz/Sparkle	0.98 ± 0.09
Sparkle/*brz*	0.30 ± 0.07
dgl + DGV/DGV	0.90 ± 0.10

dons and then on photosynthetic activity of the new leaves might be involved in this response. To force the roots to receive their nutrition from photosynthetic tissues at an earlier age, cotyledons were excised at their point of attachment to the shoot (with a razor blade) on d 9. Relative to untreated plants, root Fe(III) reductase activity was significantly enhanced in both *dgl* growth types (Fig. 2).

DISCUSSION

General Characterization

As shown previously in the *brz* pea mutant (Grusak et al., 1990a), root Fe(III) reductase activity attained elevated rates in the *dgl* pea mutant whether it was grown with or without Fe (Fig. 1). This was in contrast to its parent genotype, DVG, which exhibited a classic Fe deficiency-induced stimulation in root Fe(III) reductase activity (i.e. strategy I; Römheld and Marschner, 1986) beginning on d 15. The elevated activity in *dgl* was the result of the plasmalemma-localized Fe(III) reductase, because soluble reductant release could account for no more than 4% of total activity (see "Results"). Furthermore, agarose-BPDS studies demonstrated that functional reductase enzyme was localized throughout most of the *dgl* root system (except for root apices), as previously shown in the *brz* mutant (Grusak et al., 1990a, 1993), and similarly shown in roots of −Fe-treated DGV (d 15 plants) in this study. Because the root Fe(III) reductase previously has been argued to be the rate-limiting process in overall root Fe acquisition in pea (Grusak et al., 1990b), we conclude that the elevated reductase activity measured in the *dgl* mutant (Fig. 1), along with its extensive spatial localization, can account for the Fe-hyperaccumulating phenotype of this mutant (Kneen et al., 1990).

Shoot Regulation of Root Activity

Because root Fe(III) reductase activity in the *dgl* mutant was elevated in plants maintained on either +Fe- or −Fe nutrition (Fig. 1), it was apparent that the regulation of this process was due to some internal factor. Recently, it was shown that wild-type pea (cv Sparkle), while maintained on an Fe source, could exhibit elevated rates of root Fe(III) reduction during the period of seed development (Grusak, 1995); this root activity was regulated by a phloem-mobile signal originating in the shoot. Similarly, our results with reciprocal grafts between mutant and wild-type tissues have demonstrated that the *dgl* mutation enables the expression of a shoot factor that can promote the enhancement of Fe(III) reductase activity in wild-type roots (Table II), even when the plants are supplied with sufficient levels of Fe. We hypothesize that the *dgl* shoot transmits a signal compound that acts as a promoter in this root response; we do not believe that the mutant shoots lack a repressor signal, because the double-shoot graft combination (both *dgl* and DGV) also exhibited an elevated rate of Fe(III) reduction in wild-type rootstocks (Table II). If the mutant shoot lacked the expression and/or transmission of a repressor signal (i.e. one that signaled Fe-sufficiency status of the shoots), the DGV shoot in this double-shoot graft combination presumably would have been able to provide that signal to the roots, and the roots would have exhibited low Fe(III) reductase activity.

The existence of a promotive signal molecule is further supported by the gradual time-dependent increase in root Fe(III) reductase activity seen in both *dgl* growth types (Fig. 1). Lower rates on d 10 and 11 presumably were due to the fact that the root system had not yet received the shoot signal. Apparently, the cotyledons were the primary source of organic nutrients for the root system until about d 11 or 12, because removal of the cotyledons on d 9 resulted in an early enhancement of root Fe(III) reductase activity in the *dgl* mutant (Fig. 2). Cotyledon removal per se was not the cause of this response, because reductase activity was not significantly affected in the +Fe-treated DGV controls. These results also indicate that the signal compound is either not synthesized in, or translocated from, the cotyledons and that the initially low rates observed in *dgl* roots were not due to developmental immaturity of the root

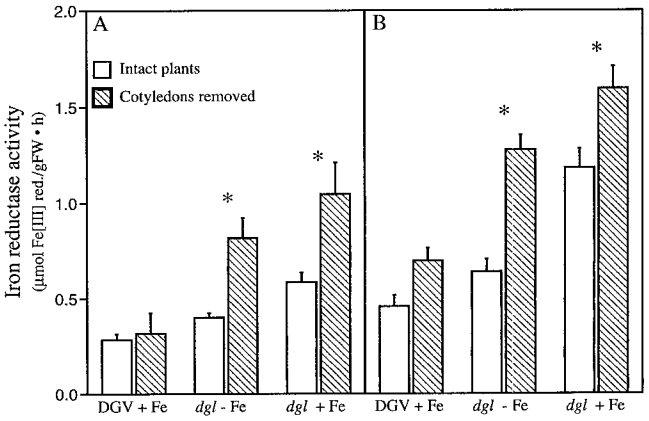

Figure 2. Influence of cotyledon removal on root Fe(III) reductase activity measured in excised root systems of +Fe-treated and −Fe-treated pea plants. +Fe-treated plants were maintained on 2 μM Fe(III)-EDDHA during growth; assays were conducted using 0.1 mM Fe(III)-EDTA. Cotyledons were excised from selected seedlings on d 9 and assays were performed on d 11 (A) or d 12 (B). Bars represent SE, $n = 4$; asterisks denote statistical differences at $P \leq 0.01$. FW, Fresh weight.

system. With the proper stimulus, even young roots could exhibit elevated reductase rates (Fig. 2). Furthermore, because cotyledons were the only source of Fe for −Fe-treated plants, the delayed increase in reductase activity seen in −Fe-treated *dgl* (relative to +Fe-treated *dgl*) might be explained by a slower transition from cotyledons to shoot tissues as sources of nourishment for those roots.

The elevated rate of root Fe(III) reduction observed in the *brz* mutant (Grusak et al., 1990a; self-grafts, Table II) was also the result of a shoot-specific factor, as shown by grafting studies (Table II). This is in apparent contradiction to an earlier report in which elevated shoot Fe levels were measured in reciprocal grafts only with *brz* as the root component (Kneen et al., 1990). In that study, only six plants of each reciprocal graft combination were analyzed, apparently in a single experiment. Because we found contradictory results, the *brz*:wild-type reciprocal grafts were repeated on four separate plantings and each time our results consistently demonstrated the involvement of a promotive signal derived from the *brz* shoots. Previously, root Fe(III) reductase measurements in *brz* revealed consistently elevated rates starting at d 10 (Grusak et al., 1990a), rather than starting at d 15 as for *dgl* in the present study (Fig. 1); qualitative measurements to localize Fe(III) reductase activity have shown an increased localization along *brz* roots (relative to Sparkle) as early as d 7 (Grusak et al., 1993). Because average weights of *brz* seeds are less than those of *dgl* seeds (0.27 versus 0.40 g dry weight), the transition from cotyledons to shoots as nutritional sources for the roots (as discussed above) may occur sooner in *brz* than in *dgl*, thereby explaining the elevated root Fe(III) reductase rates in *brz* as early as d 10 or the differences in qualitative localization observed as early as d 7 (note also that 200 μM Fe[III]-EDTA was used to enhance the staining assay in that study).

Other investigators have provided evidence for long-distance communication with respect to Fe physiology, and they have proposed the involvement of signal transmission in the regulation of the root Fe(III) reductase (Landsberg, 1984; Maas et al., 1988; Romera et al., 1992). No specific regulatory activity has yet been verified for any of the putative signal substances, nor has it been established whether the signal (or signals) regulates reductase rates through de novo protein expression or activation of existing protein. Although the signal factors conditioned by the *brz* or *dgl* mutations also have yet to be identified, it should be noted that they may be different molecules because the *dgl* and *brz* genes are nonallelic (Kneen et al., 1990). The normal function of the presumed nonmutant genes, DGL and BRZ, cannot be determined from our present results.

Rhizosphere Acidification

In addition to elevated rates of root Fe(III) reduction, an additional phenomenon associated with Fe-deficient strategy I plants is a stimulated rate of net proton efflux (Römheld and Marschner, 1986; Kochian, 1991). The resultant reduction in rhizosphere pH, with an increased solubility of Fe, is thought to be a functional strategy in response to Fe-deficiency stress (Römheld and Marschner, 1986); in pepper (*Capsicum annuum* L.), the timing of this response has been shown to correspond directly with the enhancement of root Fe(III) reductase activity (Landsberg, 1986). Elevated rates of net proton efflux were also observed with *dgl* roots (cf. +Fe-treated DGV, Table I), but the proton efflux response preceded the root Fe(III) reductase stimulation. Net proton efflux was high in both *dgl* growth types during the d 10 to 11 measurement period, a time when reductase rates were low (cf. Fig. 1) and when the root system had not yet received the shoot signal (based on cotyledon removal experiments, Fig. 2). This uncoupling of the two responses (proton efflux and reductase activity) implies that the shoot-derived signal molecule in *dgl* is not responsible, or at least is not essential, for the observed proton efflux response. Whether this shoot-derived signal molecule in *dgl* has an additive effect seems unlikely because among the *dgl* growth types only −Fe-treated *dgl* showed a significantly higher net proton efflux during the d 14 to 15 period, relative to the d 10 to 11 period (Table I). This suggests that the *dgl* shoots may be capable of generating both an Fe-deficiency signal and a novel mutant signal, with only the Fe-deficiency shoot signal promoting further enhancements in proton efflux in the roots.

The fact remains, however, that *dgl* roots had higher rates of proton efflux than DGV roots during the d 10 to 11 period. This would argue that the *dgl* gene is expressed in roots but that this expression in root tissues has an alternative physiological consequence to expression in the shoots. Furthermore, it then would follow that the *dgl* gene product(s) is not the shoot signal compound itself but possibly a regulatory protein or a biosynthetic enzyme that leads to signal generation in the shoots and proton efflux stimulation in the roots. The *dgl* gene product may alter or interfere with a normal component of a signal transduction mechanism regulating Fe homeostasis in plants. We are currently attempting to identify the product(s) of this gene expression in various tissues of the mutant.

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